

THE SPIKELET CALLUS OF *ERIOCHLOA VILLOSA* (POACEAE)

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ABSTRACT

The spikelet callus morphology of *Eriochloa villosa* (Thunb.) Kunth is examined and compared with that in other species of the genus. The callus is divided into two parts, a lower hardened bead and an upper fleshy cup. The cup possesses a membranous ventral projection which often surpasses the cup apex. Epidermal characteristics of the callus are similar to those of most other species in the genus. The cup and ventral projection contain substantial amounts of lipids which may act to attract animal dispersal agents.

RESUMEN

La morfología del callo de la espiguilla de *Eriochloa villosa* (Thunb.) Kunth se examinó y se comparó con la de otras especies del género. El callo se divide en dos partes, una endurecida, talón inferior, y una taza carnosa superior. La copa posee una proyección ventral membranosa que a menudo supera el ápice de la taza. Las características epidérmicas de los callos son similares a los de la mayoría de otras especies del género. La copa y la proyección ventral contienen lípidos que pueden actuar para atraer a agentes animales de dispersión.

INTRODUCTION

The panicoid grass genus *Eriochloa* contains about 30–35, primarily tropical and subtropical, species which are characterized by an unusual swelling and cup-like callus structure at the base of the spikelets (Clayton & Renvoise 1986). This unusual structure has been interpreted as a swelling of the lowest rachilla internode surmounted by the lower (proximal) glume which is reduced to a short flange of tissue (Hsu 1965; Shaw & Smeins 1979, 1983). However, in their anatomical examination of the callus of three *Eriochloa* species, Thompson et al. (1990) found that the stele in the spikelet rachis remained unbranched until the upper glume traces, and interpreted the lack of stelar nodes within the callus region as an indication that no part of the callus is likely to represent a vestigial lower glume. Although the callus structure has been investigated in a number of *Eriochloa* species, it has not been studied in *E. villosa* (Thunb.) Kunth. This species is native to eastern Asia from the Russian Far East to northern Viet Nam, but has been introduced to other areas including North America where it has been spreading as an agricultural weed (Darbyshire et al. 2003).

Shaw and Smeins (1979, 1983) examined epidermal characteristics of the spikelet callus in 20 species of *Eriochloa*. They observed that the structure was divided into two parts, both with characteristics of the epidermis consistent with the interpretation that the callus is formed from an expansion of the lowest rachilla internode and surmounted by the remnants of the first glume. Three types of calluses were described (Table 1). The Type 1 callus was by far the most common in the genus, being seen in 16 of the species examined (*E. aristata* Vasey, *E. boxiana* Hitchc., *E. contracta* Hitchc., *E. eggersii* Hitchc., *E. ekmanii* Hitchc., *E. acuminata* (J. Presl) Kunth (= *E. lemmonii* var. *gracilis* (E. Fourn.) Gould), *E. lemmonii* Vasey & Scribn., *E. meyeriana* (Nees) Pilger, *E. michauxii* (Poir.) Hitchc., *E. montevidensis* Griseb., *E. pacifica* Mez, *E. peruviana* Mez, *E. punctata* (L.) Ham., *E. sericea* (Scheele) Vasey, *E. setosa* (A. Rich.) Hitchc., and *E. weberbaueri* Mez). Distinctive features of the Type 1 callus included a basal portion with a smooth epidermis beset with silica bodies and an apical portion of plicate epidermal tissue. The Type 2 callus, seen in 3 species (*E. distachya* Kunth, *E. grandiflora* (Trin.) Benth.

TABLE 1. Comparison of *Eriochloa* callus characteristics described by Shaw and Smeins (1979) with those observed on *E. villosa*.

Character	Callus Type 1	Callus Type 2	Callus Type 3	<i>E. villosa</i>
bead texture	smooth	smooth	"roughened mosaic"	smooth
bead epidermis	smooth	smooth	rough	smooth to minutely roughened
bead macrohairs	absent	absent	absent	present; occasional
bead microhairs	absent	absent	absent	present (distal part)
bead stomata	absent	absent	absent	absent
bead silica bodies	present; bilobed and tetralobed	absent	absent	present (proximal part); rounded, elliptical or weakly lobed
cup texture	heavily plicate	heavily plicate	lightly plicate	heavily plicate
cup macrohairs	absent	absent	absent	present; occasional
cup microhairs	present	absent	present	present
cup stomata	absent	absent	absent	absent
cup silica bodies	absent	absent	present; trilobate, tetralobate	absent

and *E. nelsonii* Scribn. & J.G. Sm.), was similar to Type 1 but larger in size and lacking silica bodies. The Type 3 callus, seen in only one New World species (*E. polystachya* Kunth), had the basal portion without a smooth and indurate epidermis and the apical portion was a membranous "reduced glume" encircling the base of the spikelet. The Old World species *E. roxburghii* (Pilg.) Clayton (= *E. biglumis* Clayton) is somewhat anomalous in the genus, possessing a swollen spikelet base and well-developed lower glume, although Shaw and Smeins (1983) state that the lower glume is similar to that seen in *E. polystachya*.

The purpose of this study was to examine the characteristics of the spikelet callus in the Asian *E. villosa* and compare it with the previously published observations on other species which are primarily from North and South America and Africa.

MATERIALS AND METHODS

Spikelets originating from populations occurring in southern Quebec (Darbyshire et al. 2003) were used. Thirty randomly selected spikelets from one population were measured under 10× magnification for spikelet and callus size and the measurements were compared with those given by Shaw and Smeins (1979). Selected spikelets were air-dried, coated with gold and examined with a Philips XL30 ESEM microscope at 5kV acceleration voltage. Spikelets stored in 70% ethanol were re-hydrated, hand sectioned and stained with methylene blue or toluidine blue O. Air-dried spikelets were re-hydrated, hand sectioned and stained with Sudan IV.

RESULTS AND DISCUSSION

Spikelets are broadly elliptical and dorsiventrally compressed (Fig. 1 A). The size is reported as 3.9–5.5(–6.5) mm long, 2.0–2.8(–3.0) mm wide (Darbyshire et al. 2003; Shaw et al. 2003). Spikelets from the Quebec population measured 5.3 (SD = 0.18) mm long and 2.7 (SD = 0.09) mm wide, with the callus 0.8 (SD = 0.09) mm long and 1.5 (SD = 0.12) mm wide. The observed *E. villosa* callus size was larger than in any of the 19 species examined by Shaw and Smeins (1979). The callus length and width averaged 15% and 55% of the spikelet length and width, respectively. Shaw and Smeins (1979) reported variation in callus sizes between species they measured, with Type 2 calluses tending to be larger than Type 1, but no correlation between callus size (length/width) and type was detected in this study (data not shown). Epidermal characteristics of the callus of *E. villosa* are most similar to those described as Type 1 by Shaw and Smeins (1979).

Apart from the central bundles, no branching of vascular tissue was observed anywhere in the callus structure (cf. Thompson et al. 1990). The callus was divided into two more or less equal and strongly demarcated parts (Fig. 1). The basal part (bead) is formed of hardened tissues and the apical portion (cup) above is fleshy. The cup of the callus usually did not form a complete ring thereby incompletely clasping the spikelet (Fig. 1 B), although sometimes the cup was almost completely encircling with only a small notch at the junction

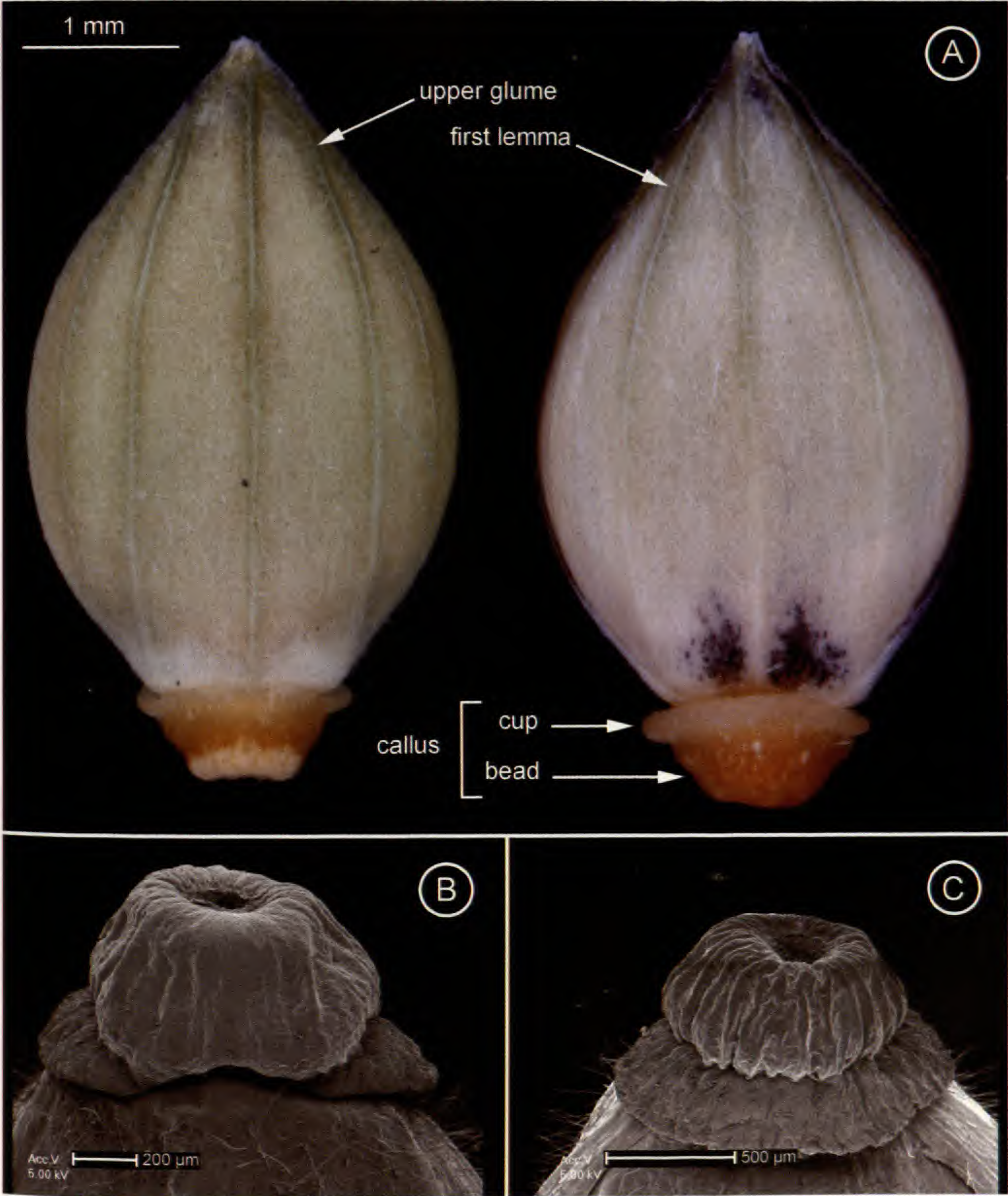


FIG. 1. Spikelets of *Eriochloa villosa*. A, left adaxial view, right abaxial view (light); B, adaxial view of spikelet callus (SEM); C, abaxial view of spikelet callus (SEM).

of the lateral edges (Fig. 1 A, left). The callus cup was directly opposite the upper glume or only slightly off centre (i.e., not quite bilaterally symmetrical). An additional flap of tissue arising from the adaxial surface of the callus cup is present in some species of *Eriochloa* and was referred to as an “extension” by Shaw and Smeins (1979) and as a “ventral projection” by Thompson et al. (1990). The ventral projection in the callus of *E. villosa* was observed to arise from the adaxial side of the cup at its base, where it formed a second inner, but incomplete

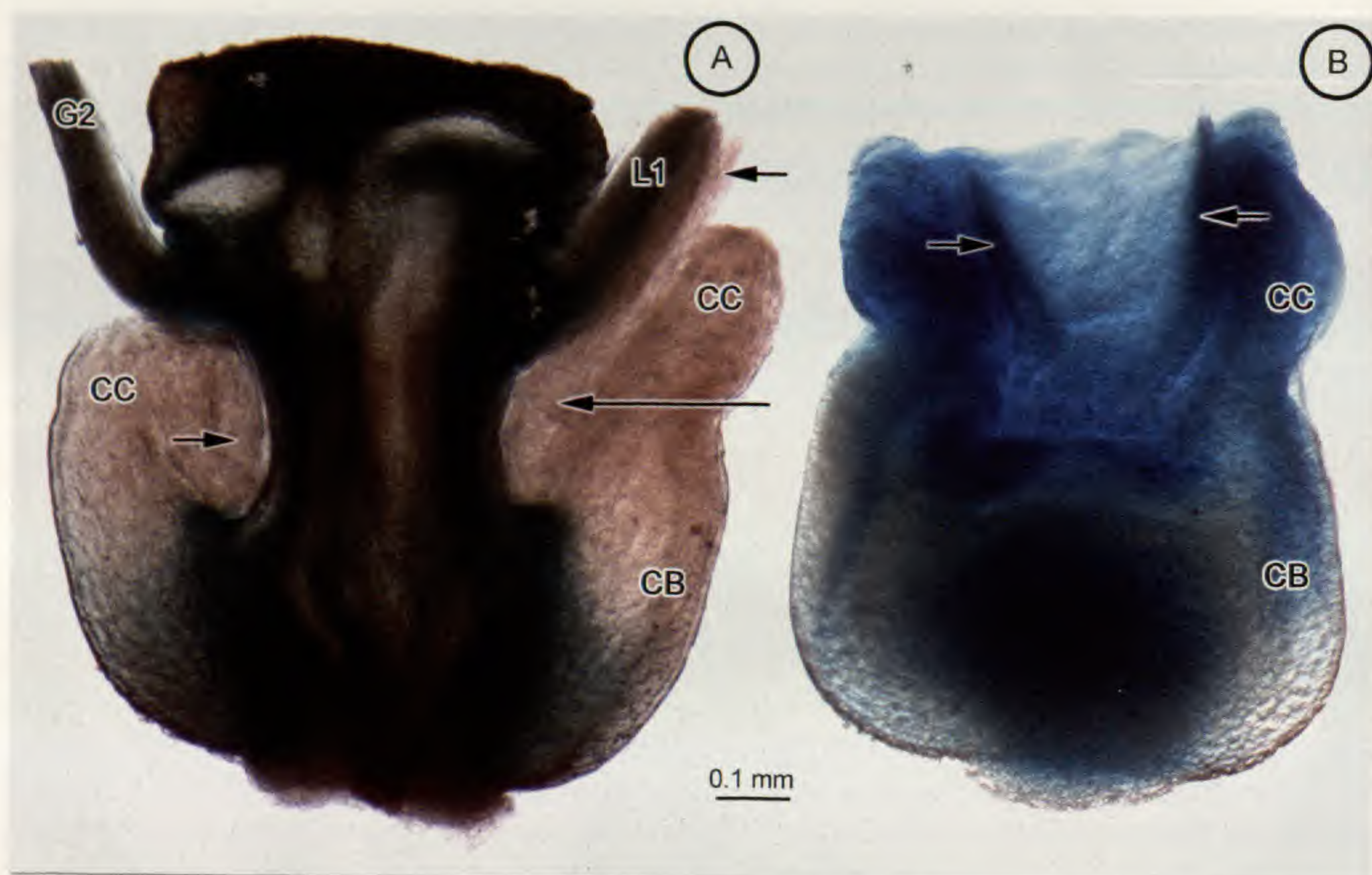


FIG. 2. Spikelet callus of *Eriochloa villosa*. **A**, longitudinal section (approximately medial) stained with toluidine blue 0 showing poorly stained ventral projection (arrows) extending beyond callus cup in the central region (abaxial side on the right) and reduced in the marginal region (adaxial side on the left); **B**, tangential plane section stained with methylene blue showing ventral projection (arrows). CB = callus bead; CC = callus cup; G2 = upper glume; L1 = lower lemma.

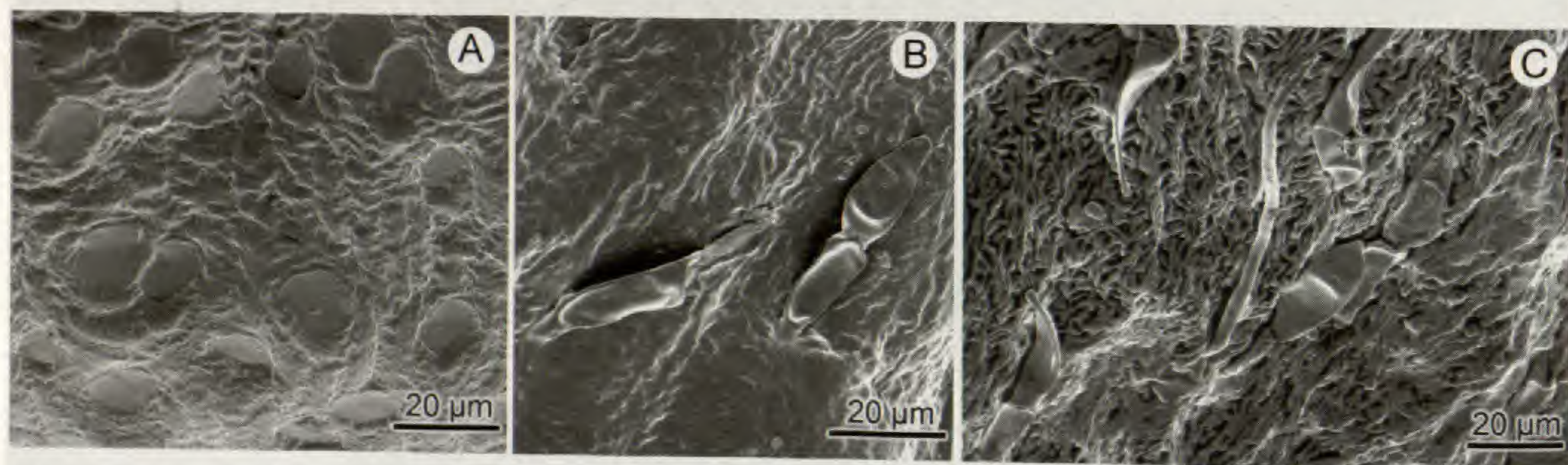


FIG. 3. Scanning electron micrographs of *Eriochloa villosa* spikelet callus. **A**, proximal callus bead showing rounded silica bodies; **B**, distal callus bead showing bicellular microhairs; **C**, callus cup showing plicate epidermis, a single macrohair (center) and several bicellular microhairs.

membranous cup (Fig. 2 A, B). Sometimes the ventral projection extended beyond the fleshy portion of the cup (Fig. 1 A (right), 2 A), but usually this flange of tissue was not visible without dissection (Fig. 2 B, 4). The position of the ventral projection is consistent with the position of the first glume, although there is no other evidence of homology.

No stomata or prickly hairs were observed on either part of the *E. villosa* callus. The bead epidermis was hardened and smooth or minutely roughened (Fig. 3 A, B). Bicellular microhairs were common apically (Fig. 3 B), but macrohairs were rarely seen. Rounded to broadly elliptic or shallowly lobed silica cells were common basally (Fig. 3 A). The abaxial epidermis of the cup was heavily plicate (Fig. 3 C). Silica bodies were not observed on the cup. Bicellular microhairs were common throughout the cup and macrohairs were occasionally

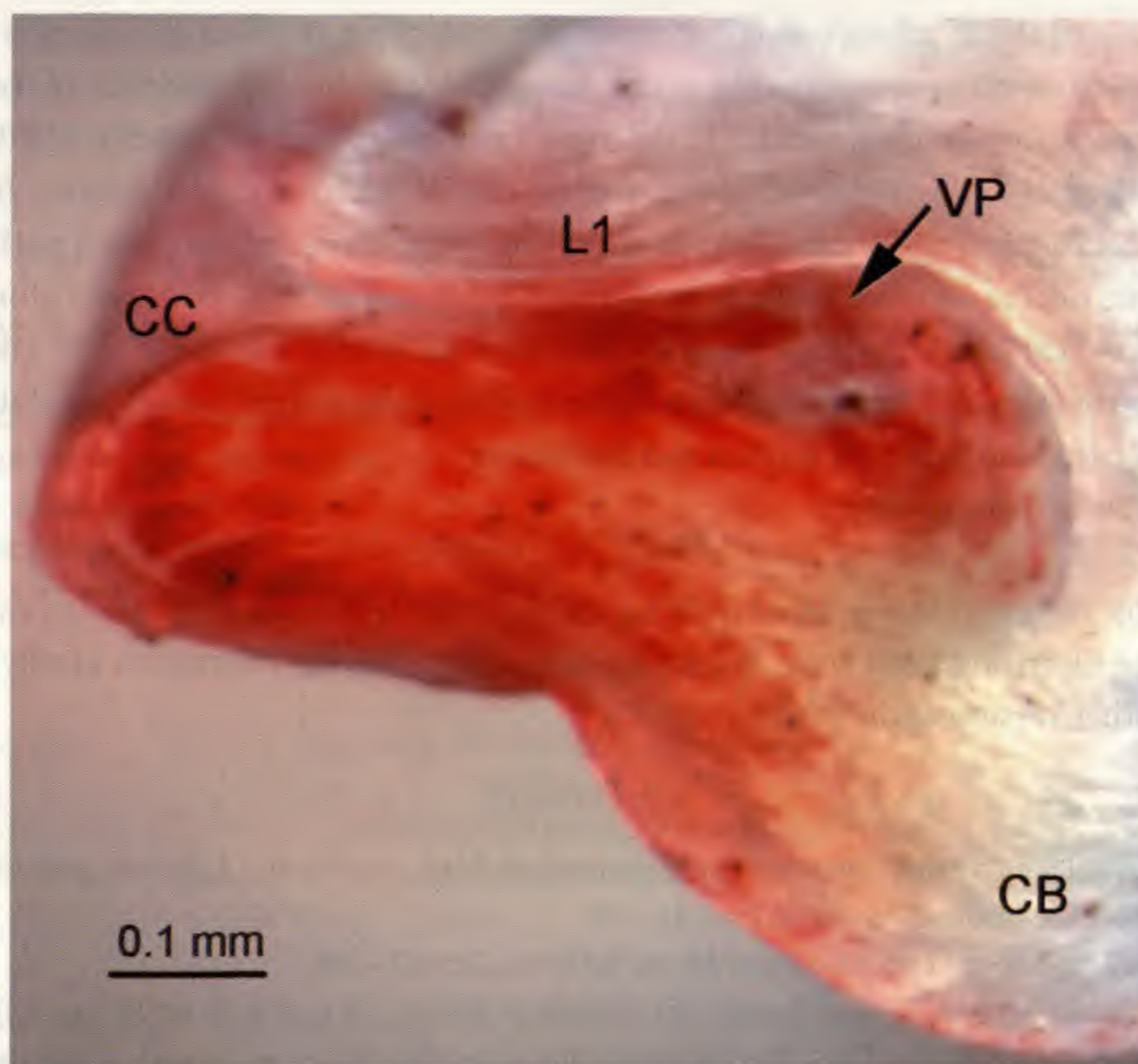


FIG. 4. Longitudinal section of *Eriochloa villosa* spikelet callus, stained with Sudan IV. CB = callus bead; CC = callus cup; L1 = lower lemma; VP = ventral projection. In this callus the ventral projection extends only about half the distance of the cup (apex just to the lower left of the "L1" annotation).

seen (Fig. 3 C). Long cells with interlocking cell walls were seen in the epidermis of the cup and the ventral projection.

Unlike the callus descriptions of most other *Eriochloa* species, occasional macrohairs were detected on both the lower and apical portions of the *E. villosa* callus (Table 1; Fig. 3 C), but see also Shaw and Smeins (1983). This is not unexpected as macrohairs are a common epidermal feature on other structures of *E. villosa* (cf., Thompson et al. 1990) and their occasional detection simply a function of the large numbers of *E. villosa* calluses examined.

The callus of untreated spikelets has an oily or waxy appearance (Fig 1A). Parenchyma cells of the cup portion and the ventral projection of the callus contained large vacuoles whose contents stained red with Sudan IV, indicating that this tissue is rich in lipids (Fig. 4). A lesser amount of staining occurred in the upper portion of the harder bead tissues of the callus. The large amount of lipids present in the fleshy callus cup suggests that this tissue may act as an elaiosome (myrmecochory) or animal attractant (Davidse 1987). No evidence was observed of oil secretion or accumulation in concave cavity formed by the cup (or ventral projection), as was suggested by Arriaga (2000). It is uncertain what types of vectors might serve as effective dispersal agents, but various types of insect and vertebrate (including birds and rodents) seed predators might be attracted to the lipid food source. Optimal seedling emergence was observed from soil depths up to about 5 cm, but occurred from depths > 9 cm (Liu & Owen 2003). This would suggest that diaspores can tolerate burial by seed caching animals.

The specific gravity of plant oils typically range from 0.91 to 0.97 at 15°C (Lide 1990). Large quantities of oils may affect buoyancy, movement and orientation (when unevenly distributed) of *E. villosa* diaspores under aqueous conditions. While the callus cup is unlikely to have a major impact on hydrochory, the increased buoyancy at the basal portion of the spikelet may provide some secondary functionality in diaspore transport and placement when free water is present.

The observations of the *E. villosa* callus are consistent with those of Shaw and Smeins (1979, 1983) and Thompson et al. (1990) on other species in the genus, but provide no further evidence of the ontogeny of the spikelet callus tissues. Although most similar to the Type 1 callus of Shaw and Smeins (1979), the most common type, slight differences were observed in the distribution of micro- and macrohairs. In most species of *Eriochloa* the lower glume is usually described as absent or greatly reduced and fused with the callus bead, however some species or taxa ascribed to the genus are said to have well-developed lower glumes and resemble species of *Brachiaria* (Clayton 1975; Gibbs Russell 1981; Shaw & Smeins 1983). Detailed anatomical study of these species (primarily African) is necessary for a better understanding of the *Eriochloa* callus homologies and the generic relationships of *Eriochloa*, *Urochloa* and *Brachiaria*.

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